PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 17 November 2000 (17.11.00)	
International application No. PCT/GB00/01190	**Applicant's or agent's file reference** 27.68545/001
International filing date (day/month/year) 28 March 2000 (28.03.00)	**Priority date** (day/month/year) 29 March 1999 (29.03.99)
Applicant GOLDSBOROUGH, Andrew	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 October 2000 (13.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

REC'D 14 JUN 2001
IPO PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
27.68545/001.hd		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01190	28/03/2000	29/03/1999
International Patent Classification (IPC) or national classification and IPC C07H21/00		
Applicant GOLDSBOROUGH, Andrew		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/10/2000	12.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vogt, T Telephone No. +49 89 2399 8477

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01190

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17,19-27, 29-36	as originally filed			
18,28	as received on	23/05/2001	with letter of	21/05/2001

Claims, No.:

1-27	as received on	23/05/2001	with letter of	21/05/2001

Drawings, sheets:

1/2,2/2	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 19, 20 .

because:

   ☒ the said international application, or the said claims Nos. 19, 20 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☒ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-18, 21-27.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13, 18, 21, 27
	No:	Claims	14-17, 22-26
Inventive step (IS)	Yes:	Claims	1-13, 21, 27
	No:	Claims	14-18, 22-26
Industrial applicability (IA)	Yes:	Claims	1-27
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

I Amendments (Art. 19(2), 34(2) PCT).

The applicant filed a new set of claims based on a combination of claim 5 with claims 1, 15, 16 and 23 as originally filed. Claim 5 was deleted and the remaining claims renumbered accordingly.

The applicant amended claims 19 and 20 (20 and 21 as originally filed) in such a way that they no longer refer to other claims. The term 'preferably' is not considered to be restrictive.

The applicant omitted the term 'etc' from the description on p. 18 and 28 as requested.

IV Lack of unity of invention (Rule 13 PCT).

Amended claims 19 and 20 are now formulated as independent claims.

The linking concept between said claims and independent claims 1, 14, 15 and 22 is an oligonucleotide (nucleic acid, nucleotide linker) characterised in that it comprises an unconventional nucleic acid at a pre-determined site.

This concept, however, is disclosed by D1, D2 and D4 (see point V for further explanation).

Hence, the examining authority is of the opinion that amended claims 19 and 20 lacks unity of invention with the remaining claims. (Rule 13 PCT).

The reasoned statement will focus on the subject matter of the remaining claims.

V Reasoned Statement (Rule 66(2) PCT).

Subject matter of the present application.

The subject matter of the present application is the provision of a method for detaching a nucleic acid from a solid support, wherein said nucleic acid contains an unconventional nucleotide at a predetermined site, and said nucleic acid is enzymatically cleaved at the site of the unconventional nucleotide using a DNA-

glycosylase specific for said unconventional nucleotide.

Cited prior art documents. (Rule 64(1) PCT).

D1: US-A-5700642.
D2: WO-A-9209615.
D3: US-A-5367066.
D4: MAG ET AL. (1991) NUCLEIC ACIDS RESEARCH 19, 1437-1441.
D5: US-A-4775619 (cited in D3).

D1 discloses a primer comprising a cleavable site. Wherein the cleavable site can be a ribonucleotide in an oligo-deoxyribonucleotide (col. 7, l. 65; example 3). This primer can be bound to biotin (col. 9, l. 41) which can be immobilized to magnetic beads modified with streptavadin (examples 2, 3 and 5).

D2 discloses a method for the synthesis of oligo nucleotides, characterized in that the first nucleotides is bound via a silyl ester bond to the solid support.

D3 discloses a modified polynucleotide containing at least one cleavable or a-basic site. In example 4 D3 discloses the synthesis of an oligonucleotide attached to a solid support comprising a modified light susceptible nucleotide. In this example said oligonucleotide is first detached from the solid support and then cleaved using light irradiation and subsequent treatment with NaOH (see scheme 6).

D4 discloses a method for the selective cleavage of an oligonucleotide from a solid support. Said method comprises the incorporation of a 3'-O-P-S-5' bond at a pre-determined position in the oligonucleotide and the selective cleavage thereof by $AgNO_3$ (see Summary and Conclusion, p. 1440).

Novelty. (Art. 33(2) PCT).

Because the novelty of the subject matter of claim 5 was previously acknowledged, amended independent claim 1 and all the claims dependent thereon (2-13 and 21) inherently meet the requirements of novelty.

D1 discloses a ribonucleotide in an oligo-deoxyribonucleotide to establish a selective cleavage site in a linker. Such a linker can be chemically cleaved (as in D1) but also

enzymatically cleaved (cf. claim 4). The subject matter of claims 14-17 and 22-26 does, therefore, not meet the requirements of novelty over D1.

Inventive step. (Art. 33(3) PCT).

The concept of selective cleavage of an oligonucleotide at the site of an unconventional or an 'a-basic' nucleotide is already known from the prior art documents. The present application modified this procedure to obtain the possibility to select between oligo-nucleotides based on the combined application of an 'unconventional' nucleotides and the selectivity of enzymes therefor (cf. claims 21 and 28). Said DNA-glycosylase performs the selective step in the cleavage process by creating an 'a-basic' nucleotide. This is then followed by an unselective 'known' step of degrading the bond between the formed 'a-basic' nucleotide and the next nucleotide. The methods disclosed in the prior art do not have this selective possibility.

Although the nucleotides of claim 4 and the corresponding DNA glycosylases are already known from the prior art (see p. 8 of the description), there is no indication in the available prior art that suggests the use thereof for site specific cleavage of oligonucleotides from solid supports.

Based on the above the subject matter of claims 1-13 and 21 meets the requirements of inventive step. (Art. 33(3) PCT).

The examining authority is of the opinion that a skilled artisan would interpret the teaching of D1 in a way that any biological relevant molecule (marker, ligand, antibody, substrate, inhibitor, etc.) can be coupled to the linker of D1. The examining authority is therefore of the opinion that the subject matter of claim 18 does not meet the requirements of inventive step. (Art. 33(3) PCT).

Industrial applicability. (Art. 33(4) PCT).

The oligonucleotides and the method of cleavage the same have a wide range of applicability. For instance, in the diagnosis of diseases and bacterial infections. Based on the above the subject matter of the present application meets the requirements of industrial applicability. (Art. 33 (4) PCT)

VIII Clarity of the claims. (Art. 6 PCT).

Lack of clarity of the claims as a whole arises, because the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, independent claims 14, 15, 19, 20, 21, 22 and 27 do not meet the requirements of Article 6 PCT. The applicant should file an amended set of claims wherein said claims refer to claim 1 insofar as the same subject matter is concerned.

The terms 'a construct' and 'a functional group' used in claims 14 and 15 are vague and unclear and leave the reader in doubt as to the meaning of the technical feature to which they refer, thereby rendering the definition of the subject-matter of said claims unclear. (Art. 6 PCT).

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DZIEGLEWSKA, Hanna
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

FILE 68545/01L
14 JUN 2001
RE
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ANSD

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	12.06.2001
Applicant's or agent's file reference 27.68545/001.hd	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01190	28/03/2000	29/03/1999

Applicant
GOLDSBOROUGH, Andrew

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Gallego, A Tel.+49 89 2399-8102

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
27.68545/001.hd		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01190	28/03/2000	29/03/1999
International Patent Classification (IPC) or national classification and IPC C07H21/00		
Applicant GOLDSBOROUGH, Andrew		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/10/2000	12.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vogt, T Telephone No. +49 89 2399 8477

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01190

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17,19-27, 29-36	as originally filed			
18,28	as received on	23/05/2001	with letter of	21/05/2001

Claims, No.:

1-27	as received on	23/05/2001	with letter of	21/05/2001

Drawings, sheets:

1/2,2/2	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 19, 20 .

because:

   ☒ the said international application, or the said claims Nos. 19, 20 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☒ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-18, 21-27.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13, 18, 21, 27
	No:	Claims	14-17, 22-26
Inventive step (IS)	Yes:	Claims	1-13, 21, 27
	No:	Claims	14-18, 22-26
Industrial applicability (IA)	Yes:	Claims	1-27
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

I Amendments (Art. 19(2), 34(2) PCT).

The applicant filed a new set of claims based on a combination of claim 5 with claims 1, 15, 16 and 23 as originally filed. Claim 5 was deleted and the remaining claims renumbered accordingly.

The applicant amended claims 19 and 20 (20 and 21 as originally filed) in such a way that they no longer refer to other claims. The term 'preferably' is not considered to be restrictive.

The applicant omitted the term 'etc' from the description on p. 18 and 28 as requested.

IV Lack of unity of invention (Rule 13 PCT).

Amended claims 19 and 20 are now formulated as independent claims.

The linking concept between said claims and independent claims 1, 14, 15 and 22 is an oligonucleotide (nucleic acid, nucleotide linker) characterised in that it comprises an unconventional nucleic acid at a pre-determined site.

This concept, however, is disclosed by D1, D2 and D4 (see point V for further explanation).

Hence, the examining authority is of the opinion that amended claims 19 and 20 lacks unity of invention with the remaining claims. (Rule 13 PCT).

The reasoned statement will focus on the subject matter of the remaining claims.

V Reasoned Statement (Rule 66(2) PCT).

Subject matter of the present application.

The subject matter of the present application is the provision of a method for detaching a nucleic acid from a solid support, wherein said nucleic acid contains an unconventional nucleotide at a predetermined site, and said nucleic acid is enzymatically cleaved at the site of the unconventional nucleotide using a DNA-

glycosylase specific for said unconventional nucleotide.

Cited prior art documents. (Rule 64(1) PCT).

D1: US-A-5700642.
D2: WO-A-9209615.
D3: US-A-5367066.
D4: MAG ET AL. (1991) NUCLEIC ACIDS RESEARCH 19, 1437-1441.
D5: US-A-4775619 (cited in D3).

D1 discloses a primer comprising a cleavable site. Wherein the cleavable site can be a ribonucleotide in an oligo-deoxyribonucleotide (col. 7, l. 65; example 3). This primer can be bound to biotin (col. 9, l. 41) which can be immobilized to magnetic beads modified with streptavadin (examples 2, 3 and 5).

D2 discloses a method for the synthesis of oligo nucleotides, characterized in that the first nucleotides is bound via a silyl ester bond to the solid support.

D3 discloses a modified polynucleotide containing at least one cleavable or a-basic site. In example 4 D3 discloses the synthesis of an oligonucleotide attached to a solid support comprising a modified light susceptible nucleotide. In this example said oligonucleotide is first detached from the solid support and then cleaved using light irradiation and subsequent treatment with NaOH (see scheme 6).

D4 discloses a method for the selective cleavage of an oligonucleotide from a solid support. Said method comprises the incorporation of a 3'-O-P-S-5' bond at a pre-determined position in the oligonucleotide and the selective cleavage thereof by $AgNO_3$ (see Summary and Conclusion, p. 1440).

Novelty. (Art. 33(2) PCT).

Because the novelty of the subject matter of claim 5 was previously acknowledged, amended independent claim 1 and all the claims dependent thereon (2-13 and 21) inherently meet the requirements of novelty.

D1 discloses a ribonucleotide in an oligo-deoxyribonucleotide to establish a selective cleavage site in a linker. Such a linker can be chemically cleaved (as in D1) but also

enzymatically cleaved (cf. claim 4). The subject matter of claims 14-17 and 22-26 does, therefore, not meet the requirements of novelty over D1.

Inventive step. (Art. 33(3) PCT).

The concept of selective cleavage of an oligonucleotide at the site of an unconventional or an 'a-basic' nucleotide is already known from the prior art documents. The present application modified this procedure to obtain the possibility to select between oligo-nucleotides based on the combined application of an 'unconventional' nucleotides and the selectivity of enzymes therefor (cf. claims 21 and 28). Said DNA-glycosylase performs the selective step in the cleavage process by creating an 'a-basic' nucleotide. This is then followed by an unselective 'known' step of degrading the bond between the formed 'a-basic' nucleotide and the next nucleotide. The methods disclosed in the prior art do not have this selective possibility.

Although the nucleotides of claim 4 and the corresponding DNA glycosylases are already known from the prior art (see p. 8 of the description), there is no indication in the available prior art that suggests the use thereof for site specific cleavage of oligonucleotides from solid supports.

Based on the above the subject matter of claims 1-13 and 21 meets the requirements of inventive step. (Art. 33(3) PCT).

The examining authority is of the opinion that a skilled artisan would interpret the teaching of D1 in a way that any biological relevant molecule (marker, ligand, antibody, substrate, inhibitor, etc.) can be coupled to the linker of D1. The examining authority is therefore of the opinion that the subject matter of claim 18 does not meet the requirements of inventive step. (Art. 33(3) PCT).

Industrial applicability. (Art. 33(4) PCT).

The oligonucleotides and the method of cleavage the same have a wide range of applicability. For instance, in the diagnosis of diseases and bacterial infections. Based on the above the subject matter of the present application meets the requirements of industrial applicability. (Art. 33 (4) PCT)

VIII Clarity of the claims. (Art. 6 PCT).

Lack of clarity of the claims as a whole arises, because the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, independent claims 14, 15, 19, 20, 21, 22 and 27 do not meet the requirements of Article 6 PCT. The applicant should file an amended set of claims wherein said claims refer to claim 1 insofar as the same subject matter is concerned.

The terms 'a construct' and 'a functional group' used in claims 14 and 15 are vague and unclear and leave the reader in doubt as to the meaning of the technical feature to which they refer, thereby rendering the definition of the subject-matter of said claims unclear. (Art. 6 PCT).

derivatives and synthetic antibodies such as single chain antibodies), an enzyme or a receptor protein or some other binding protein or binding portion or fragment thereof (e.g. streptavidin, protein A, protein G, protein L, or fragments thereof or indeed any known, synthetic or modified (e.g. genetically modified) affinity binding protein such as antibodies, lectins ~~[etc]~~). Alternatively, the linker sequence may be coupled to an enzyme substrate, a receptor ligand, an antigen/hapten or fragment thereof ~~[etc]~~. Advantageously, therefore, the "second" component of the chimeric nucleic acid molecule is an affinity binding group i.e. one of a pair of affinity binding partners.

Such chimeric nucleic acid molecules have utility in any solid phase process or procedure based on affinity binding, for example in separation and purification procedures, e.g. of cells or proteins or other molecules, or in assays.

A further aspect of the invention thus provides a method of preparing a construct for binding to, and subsequent cleavage from, a solid support, said method comprising incorporating into said construct a nucleotide sequence comprising at a pre-determined site an unconventional nucleotide capable of selective cleavage.

In a still further aspect, the present invention also provides a chimeric molecule (or construct) comprising a nucleotide linker sequence comprising a selectively cleavable unconventional nucleotide at a pre-determined site, coupled to a functional group, preferably an affinity binding group or a reporter group.

Advantageously, in such a chimeric molecule the linker sequence is further either immobilised (i.e. bound to a solid support) or provided with means for immobilisation to a solid support, as discussed above.

The functional group may be any group having a

be added to each biotinylated PCR primer. As a representative example, the forward and reverse PCR primers could each incorporate a different unconventional nucleotide. For example primer T3 may incorporate U and primer T7 may incorporate methyl adenine (MA) (i.e. $T3^{U}$ and $T7^{MA}$). Following amplification and binding to a streptavidin bead, and denaturation to separate the strands, each strand of the PCR product may then be detached in turn using glycosylase UDG or methyl adenine (MA) glycosylase. This simplifies having to prepare a separate purification tube for each strand purified.

Alternatively, multiple RT-PCR reactions could be purified together in order to compare gene expression levels. In other words, the expression levels or patterns of different genes may be compared, by using RT primers for different genes, each having a different unconventional nucleotide, which would permit each different RT product to be relatively cleaved. As a representative example of such a method, an assay can be envisaged in which, an assay for GAPDH, for example, would have a forward (or reverse) biotinylated primer containing U (e.g. GAPDH - F^{U}). Then the assay for the mRNA of interest, e.g. p53, would have biotin p53 - F^{MA}. The PCR reaction would incorporate a fluorescent deoxynucleotide into both the GAPDH and p53 PCR products, which could both be purified together in the same tube containing a biotin-binding solid support e.g. streptavidin-beads (e.g. M-280 Dynabeads from Dynal ASA, Norway). After washing ~~etc~~ to remove unincorporated nucleotides, the amount of GAPDH PCR product could be measured following the addition of UDG and analysing the amount of fluorescence released from the bead. Likewise, p53 could be measured following the addition of methyl adenine glycosylase.

The only limits to this multiplex approach are the number of different glycosylases. In the case of ten

Claims

1. A method of detaching a nucleic acid molecule from a solid support to which it is attached, wherein an unconventional nucleotide is incorporated at a pre-determined site in said nucleic acid molecule, said method comprising selectively cleaving said nucleic acid molecule at the site of said unconventional nucleotide, wherein said selective cleavage is accomplished enzymically.

2. A method of reversibly immobilising a nucleic acid molecule, said method comprising:

(a) incorporating an unconventional nucleotide into said nucleic acid molecule at a pre-determined site;

(b) binding said nucleic acid molecule to a solid support; steps (a) and (b) being carried out in either order or simultaneously and subsequently

(c) selectively cleaving said nucleic acid molecule at the site of said unconventional nucleotide, wherein said selective cleavage is accomplished enzymically.

3. A method as claimed in claim 1 or claim 2 wherein said nucleic acid molecule is a chimeric molecule comprising a nucleic acid component and another non-nucleic acid component.

4. A method as claimed in any one of claims 1 to 3, wherein the unconventional nucleotide is uracil, hypoxanthine, a ribonucleotide, N-7 methylguanine, 8-oxoguanine, deoxyuridine, deoxyinosine, deoxy 5,6-dihydroxythimine, 5'6'-dihydroxythine, deoxy 3'-methyladenosine or 3'-methyladenosine.

5. A method as claimed in any one of claims 1 to 4, wherein said selective cleavage is achieved using a DNA glycosylase enzyme.

6. A method as claimed in any one of claims 1 to 5, wherein said nucleic acid molecule comprises DNA, said unconventional nucleotide is uracil (U), and selective cleavage is achieved using a uracil DNA glycosylase enzyme (UDG).

7. A method as claimed in any one of claims 1 to 6, wherein said unconventional nucleotide is incorporated into said nucleic acid molecule as part of a linker sequence.

8. A method as claimed in claim 7 wherein said linker sequence is a primer.

9. A method as claimed in any one of claims 1 to 8, wherein said nucleic acid molecule is a primer extension product.

10. A method as claimed in any one of claims 1 to 9, wherein said support is a magnetic bead.

11. A method as claimed in any one of claims 7 to 10, wherein said linker sequence is provided with means for immobilisation to a solid support.

12. A method as claimed in any one of claims 9 to 11, wherein said nucleic acid molecule is a cDNA, or a product of an *in vitro* amplification reaction or a sequencing reaction.

13. A method as claimed in any one of claims 7, 10 or 11, wherein said nucleic acid molecule comprises a linker sequence coupled to a protein, an enzyme

substrate, a receptor ligand, an antigen or hapten, or a fragment thereof, or to an affinity binding group or a reporter group.

14. A method of preparing a construct for binding to, and subsequent cleavage from, a solid support, said method comprising incorporating into said construct a nucleotide linker sequence comprising at a pre-determined site an unconventional nucleotide capable of selective cleavage using an enzyme.

15. A chimeric molecule comprising a nucleotide linker sequence comprising at a pre-determined site an unconventional nucleotide capable of selective cleavage using an enzyme, coupled to a functional group.

16. A chimeric molecule as claimed in claim 15, wherein said functional group is an affinity binding group or a reporter group.

17. A method as claimed in claim 14, or a chimeric molecule as claimed in claim 15 or 16, wherein said linker sequence is immobilised or provided with means for immobilisation to a solid support.

18. A chimeric molecule as claimed in any one of claims 15 to 17 wherein said affinity binding group is an antibody or a fragment or derivative thereof, or a hapten.

19. A method for separating a target cell from a sample, said method comprising binding said target cell to a solid support by means of a chimeric molecule comprising a nucleotide linker sequence comprising a selectively cleavable unconventional nucleotide at a pre-determined site, coupled to a functional group, preferably as defined in any one of claims 15 to 18,

wherein said functional group is an affinity binding group which binds specifically to said cell.

20. A method of detaching a nucleic acid molecule from a solid support to which it is attached, wherein an unconventional nucleotide is incorporated at a pre-determined site in said nucleic acid molecule, said method comprising selectively cleaving said nucleic acid molecule at the site of said unconventional nucleotide, or of reversibly immobilising a nucleic acid molecule, said method comprising:

(a) incorporating an unconventional nucleotide into said nucleic acid molecule at a pre-determined site;

(b) binding said nucleic acid molecule to a solid support; steps (a) and (b) being carried out in either order or simultaneously and subsequently

(c) selectively cleaving said nucleic acid molecule at the site of said unconventional nucleotide, preferably as claimed in any one of claims 1 to 13,

or a method as claimed in claim 19,

wherein a multiplicity of different nucleic acid molecules or chimeric molecules comprising a nucleotide linker sequence comprising a selectively cleavable unconventional nucleotide at a pre-determined site, coupled to a functional group, are attached or bound to a solid support, each said different molecule incorporating a different unconventional nucleotide.

21. A kit for use in a method as defined in any one of claims 1 to 13, said kit comprising

(a) means for introducing an unconventional nucleotide into a nucleic acid molecule; and

(b) means for selective cleavage of said unconventional nucleotide, wherein said means is an enzyme.

22. A poly- or oligonucleotide incorporating an unconventional nucleotide which is selectively cleavable using an enzyme, immobilised on a solid support or carrying means for immobilisation.

23. A poly- or oligonucleotide as claimed in claim 22, being poly- or oligo dU.

24. A poly- or oligonucleotide according to claim 22 being a primer.

25. A poly- or oligonucleotide as claimed in any one of claims 22 to 24, wherein said means for immobilisation is biotin.

26. A poly- or oligonucleotide as claimed in any one of claims 22 to 24 wherein said solid support comprises magnetic beads.

27. A multiplicity of oligo- or polynucleotides as defined in any one of claims 22 and 24 to 26, wherein each different oligo- or polynucleotide incorporates a different unconventional nucleotide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
27.68545/001		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/01190	28/03/2000	29/03/1999
Applicant		
GOLDSBOROUGH, Andrew		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/01190

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 700 642 A (MONFORTE JOSEPH ALBERT ET AL) 23 December 1997 (1997-12-23) column 3, line 58 -column 4, line 38 column 10, line 47 -column 11, line 46 column 24, line 42 - line 56 examples 2 and 3 figures 1 and 2	1-4, 8-19,23, 25-27
X	WO 92 09615 A (PHARMACIA LKB BIOTECH) 11 June 1992 (1992-06-11) claim 2	1,2
X	US 5 367 066 A (HORN THOMAS ET AL) 22 November 1994 (1994-11-22) abstract	16,18,23

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2000	17/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	de Nooy, A

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MAG M ET AL: "SYNTHESIS AND SELECTIVE CLEAVAGE OF AN OLIGODEOXYNYCLEOTIDE CONTAINING A BRIDGED INTERNUCLEOTIDE 5'-PHOSPHOROTHIOATE LINKAGE" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 19, no. 7, 1991, pages 1437-1441, XP000857921 ISSN: 0305-1048 page 1440, right-hand column, paragraph 2 -----	16,18,23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/01190

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5700642 A	23-12-1997	AU 695705 B	20-08-1998
		AU 5635296 A	11-12-1996
		CA 2220418 A	28-11-1996
		CN 1191575 A	26-08-1998
		DE 69605669 D	20-01-2000
		EP 0828855 A	18-03-1998
		JP 11505127 T	18-05-1999
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WO 9209615 A	11-06-1992	AT 133684 T	15-02-1996
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		WO 9202528 A	20-02-1992

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/GB 00/01190

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 700 642 A (MONFORTE JOSEPH ALBERT ET AL) 23 December 1997 (1997-12-23) column 3, line 58 -column 4, line 38 column 10, line 47 -column 11, line 46 column 24, line 42 - line 56 examples 2 and 3 figures 1 and 2	1-4, 8-19,23, 25-27
X	WO 92 09615 A (PHARMACIA LKB BIOTECH) 11 June 1992 (1992-06-11) claim 2	1,2
X	US 5 367 066 A (HORN THOMAS ET AL) 22 November 1994 (1994-11-22) abstract	16,18,23

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2000	17/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	de Nooy, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat . Application No
PCT/GB 00/01190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MAG M ET AL: "SYNTHESIS AND SELECTIVE CLEAVAGE OF AN OLIGODEOXYNYCLEOTIDE CONTAINING A BRIDGED INTERNUCLEOTIDE 5'-PHOSPHOROTHIOATE LINKAGE" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 19, no. 7, 1991, pages 1437-1441, XP000857921 ISSN: 0305-1048 page 1440, right-hand column, paragraph 2	16,18,23

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
DZIEGLEWSKA, Hanna
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

FILE 68545/001
28 DEC 2000
RECEIVED

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	27.12.2000
Applicant's or agent's file reference: 27.68545/001.hd	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01190	28/03/2000	29/03/1999

International Patent Classification (IPC) or both national classification and IPC
C07H21/00

Applicant
GOLDSBOROUGH, Andrew

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 29/07/2001.

DUE DATES NOTED
27/3/01

Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer / Examiner Vogt, T Formalities officer (incl. extension of time limits) Gallego, A Telephone No. +49 89 2399 8102

Form PCT/IPEA/408 (cover sheet) (January 1994)

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

Description, pages:

1-36 as originally filed

Claims, No.:

1-28 as originally filed

Drawings, sheets:

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrialapplicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4, 8-18, 23-27 No; 5-7, 19-22, 28 Yes
Inventive step (IS)	Claims	1-4, 8-28 No; 5-7 Yes
Industrial applicability (IA)	Claims	1-28 Yes

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

IV Lack of unity of invention (Rule 13 PCT).

The following independent claims are identified in the present application.

Claim 1. A method for detaching a nucleic acid from a solid support.

Claim 15. A method of preparing a construct comprising an oligonucleotide.

Claim 16. A chimeric molecule comprising an oligonucleotide.

Claim 20. A method for separating a target cell from a sample comprising an oligonucleotide.

Claim 21. A method according to claims 1 or 15 or 20 wherein a multiplicity of oligonucleotide molecules are attached to a solid support.

Claim 22. A kit for preparing the oligonucleotides of claim 1.

Claim 23. An immobilized oligonucleotide.

Claim 28. A mixture of oligonucleotides of claim 23.

The linking concept between said claims is an oligonucleotide (nucleic acid, nucleotide linker) characterised in that it comprises an unconventional nucleic acid at a pre-determined site.
This concept, however, is disclosed by D1, D2 and D4 (see point V for further explanation).

The requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the following groups of claims:

1) A method for detaching a nucleic acid comprising an unconventional nucleotide from a solid support, comprising the selective cleavage of said nucleic acid at the site of said unconventional nucleotide, and a kit for preparing the same. (Claims 1-14, 21 and 22).
2) A method for the preparation of a construct, comprising incorporating an oligo-nucleotide comprising an unconventional nucleotide. (Claim 15).
3) A chimeric molecule comprising an oligonucleotide coupled to a functional group, wherein said oligonucleotide comprises an unconventional nucleotide, and a method for separating a target cell, using said chimeric molecule. (Claims 16-20 and 21).
4) A oligonucleotide comprising an unconventional nucleotide, immobilised on a solid

support or carrying means, and a mixture thereof. (Claims 23-28).

The applicant appears to be able to overcome this objection by filing an amended set of claims based on a novel and inventive linking concept. For instance: a new claim 1 which is a combination of claims 1, 4, and 6 as originaly filed (compare with claim 7 as originally filed) appears to overcome said objection. See also point VIII of this communication.

V Reasoned Statement. (Art. 33 PCT)

Subject matter of the present application.
The subject matter of the present application is the provision of a method for detaching a nucleic acid from a solid support, wherein said nucleic acid contains an unconventional nucleotide at a predetermined site, and said nucleic acid is cleaved at the site of the unconventional nucleotide using a DNA-glycosylase specific for said unconventional nucleotide.

Cited prior art documents. (Rule 64(1) PCT).
D1: US-A-5700642.
D2: WO-A-9209615.
D3: US-A-5367066.
D4: MAG M. ET AL: NUCLEIC ACIDS RESEARCH 19 (1991) 1437-1441.
D5: US-A-4775619 (cited in D3).

D1 discloses a primer comprising a cleavable site. Wherein the cleavable site can be a ribonucleotide in an oligo-deoxyribonucleotide (col. 7, l. 65; example 3). This primer can be bound to biotin (col. 9, l. 41) which can be immobilized to magnetic beads modified with streptavadin (examples 2, 3 and 5).

D2 discloses a method for the synthesis of oligo nucleotides, characterized in that the first nucleotides is bound via a silyl ester bond to the solid support.

D3 discloses a modified polynucleotide containing at least one cleavable or abasic site. In example 4 D3 discloses the synthesis of an oligonucleotide attached to a solid

support comprising a modified light susceptible nucleotide. In this example said oligonucleotide is first detached from the solid support and then cleaved using light irradiation and subsequent treatment with NaOH (see scheme 6).

D4 discloses a method for the selective cleavage of an oligonucleotide from a solid support. Said method comprises the incorporation of a 3'-O-P-S-5' bond at a pre-determined position in the oligonucleotide and the selective cleavage thereof by $AgNO_3$ (see Summary and Conclusion, p. 1440).

Novelty. (Art. 33(2) PCT)

Based on D1 the subject matter of claims 1-4, 8-14, 15-18 and 23-27 does not meet the requirements of novelty. (Art. 33 (2) PCT)

A remark to claims 3 and 16-18. Claim 3 does not meet the requirement of novelty over D1 because the wording of claims 1 and 3 does not exclude the binding of the nucleic acid via the non-nucleic acid component. The term 'functional' group in claim 16 is so broad that it also comprises biotin which is an affinity binding group.

Based on D2 the subject matter of claims 1 and 2 does not meet the requirements of novelty. (Art. 33 (2) PCT)

Based on D3 the subject matter of claims 16, 18 and 23 does not meet the requirements of novelty. (Art. 33 (2) PCT)

Based on D4 the subject matter of claims 1, 2, 16, 18 and 23 does not meet the requirements of novelty. (Art. 33 (2) PCT)

Inventive step. (Art. 33(3) PCT)

The concept of selective cleavage of an oligonucleotide at the site of an unconventional or an 'abasic' nucleotide is already known from the prior art documents. The present application modified this procedure to obtain the possibility to select between oligo-nucleotides based on the combined application of an 'unconventional' nucleotides and the selectivity of the DNA-glycosylases therefor (cf. claims 21 and 28). Said DNA-glycosylase performs the selective step in the cleavage process by creating an 'abasic' nucleotide. This is then followed by an unselective 'known' step of degrading the bond between the formed 'abasic' nucleotide and the next nucleotide. The methods disclosed

in the prior art do not have this selective possibility.

Although the nucleotides of claim 4 and the corresponding DNA glycosylases are already known from the prior art (see p. 8 of the description), there is no indication in the available prior art that suggests the use thereof for site specific cleavage of oligonucleotides from solid supports.

Based on the above the subject matter of claims 5 to 7 meets the requirements of inventive step. (Art. 33(3) PCT)

Because the method of cleavage as claimed by the applicant relies on the selectivity of the DNA-glycosylases listed on p. 8 the applicant is requested to provide evidence that said DNA-glycosylases are selective enough to distinguish between structurally related nucleotides, and that as a result of said selectivity specific oligonucleotides as meant in claims 21 and 28 can be selectively cleaved from a mixture thereof.
At present, therefore, the subject matter of claims 21 and 28 does not meet the requirement of inventive step. (Art. 33(3) PCT)

The kit of claim 22 can only be considered to be inventive when a novel and inventive linking concept is provided. At present, therefore, the subject matter of claim 22 does not meet the requirements of inventive step. (Art. 33(3) PCT)

Industrial applicability. (Art. 33(4) PCT)
The oligonucleotides and the method of cleavage the same have a wide range of applicability. For instance, in the diagnosis of diseases and bacterial infections.
Based on the above the subject matter of the present application meets the requirements of industrial applicability. (Art. 33 (4) PCT)

VII Defects in the description. (Art. 5 PCT).

The applicant is requested to omit the term 'etc' from the description on p. 18, l. 8 and 10 and p. 28, l. 30.

VIII Clarity of the claims. (Art. 6 PCT).

Lack of clarity of the claims as a whole arises, because the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, independent claims 1, 15, 16, 20, 21, 22, 23, 28 do not meet the requirements of Article 6 PCT. The applicant is requested to file an amended set of claims wherein claim 1 defines the novel and inventive linking concept (see also point IV of this communication).

The term 'unconventional nucleotide' used in claims 1, 2, 8, 15, 16, 21, 22, 23 and 28 is vague and unclear. The applicant is requested to clarify said term, for instance by the incorporation of the subject matter of claim 4 into claim 1. (Art. 6 PCT)

In claims 5-7 the applicant describes that the selective cleavage should be performed enzymatically by for instance a glycosylase. However, on p. 15 l. 12-15 and examples 1 and 5 the applicant discloses that the actual cleavage is performed by raising the temperature or the addition of exonuclease III or endonuclease IV. The glycosylase merely removes the base from the ribose moiety but does not cleave the oligonucleotide. The applicant is requested to clarify this apparent contradiction. (Art. 6 PCT)

The terms 'a construct' and 'a functional group' used in claims 15 and 16 are vague and unclear and leave the reader in doubt as to the meaning of the technical feature to which they refer, thereby rendering the definition of the subject-matter of said claims unclear. (Art. 6 PCT)